

## Cellular Basis for Improved Left Ventricular Pump Function After Digoxin Therapy in Experimental Left Ventricular Failure

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**Objectives.** The present study examined left ventricular (LV) and myocyte contractile performance and electrophysiologic variables after long-term digoxin treatment in a model of LV failure.

**Background.** A fundamental therapeutic agent for patients with chronic LV dysfunction is the cardiac glycoside digoxin. However, whether digoxin has direct effects on myocyte contractile function and electrophysiologic properties in the setting of chronic LV dysfunction remains unexplored.

**Methods.** Left ventricular and isolated myocyte function and electrophysiologic variables were examined in five control dogs, five dogs after the development of long-term rapid pacing (rapid pacing, 220 beats/min, 4 weeks) and five dogs with rapid pacing given digoxin (0.25 mg/day) during the pacing period (rapid pacing and digoxin).

**Results.** Left ventricular ejection fraction decreased in the dogs with rapid pacing compared with that in control dogs ( $30 \pm 2\%$  vs.  $68 \pm 3\%$ ,  $p < 0.05$ ) and was higher with digoxin than that in the rapid pacing group ( $38 \pm 3\%$ ,  $p = 0.038$ ). Left ventricular end-diastolic volume increased in the rapid pacing group compared with the control group ( $84 \pm 6$  ml vs.  $59 \pm 7$  ml,  $p < 0.05$ ) and remained increased with digoxin ( $79 \pm 6$  ml). Isolated

myocyte shortening velocity decreased in the rapid pacing group compared with the control group ( $37 \pm 1$   $\mu\text{m/s}$  vs.  $59 \pm 1$   $\mu\text{m/s}$ ,  $p < 0.05$ ) and increased with digoxin compared with rapid pacing ( $46 \pm 1$   $\mu\text{m/s}$ ,  $p < 0.05$ ). Action potential maximal upstroke velocity was diminished in the rapid pacing group compared with the control group ( $135 \pm 6$  V/s vs.  $163 \pm 9$  V/s,  $p < 0.05$ ) and increased with digoxin compared with rapid pacing ( $155 \pm 12$  V/s,  $p < 0.05$ ). Action potential duration increased in the rapid pacing group compared with the control group ( $247 \pm 10$  vs.  $216 \pm 6$  ms,  $p < 0.05$ ) and decreased with digoxin compared with rapid pacing ( $219 \pm 12$  ms,  $p < 0.05$ ).

**Conclusions.** In this model of rapid pacing-induced LV failure, digoxin treatment improved LV pump function, enhanced isolated myocyte contractile performance and normalized myocyte action potential characteristics. This study provides unique evidence to suggest that the cellular basis for improved LV pump function with digoxin treatment in the setting of LV failure has a direct and beneficial effect on myocyte contractile function and electrophysiologic measures.

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Cardiac glycosides such as digoxin have been used in the treatment of cardiac diseases for over 200 years (1-3). Today, digoxin is routinely administered to patients with congestive heart failure. Several recent clinical trials have demonstrated that chronic digoxin administration in congestive heart failure improves left ventricular (LV) function and reduces the sever-

ity of subjective symptoms (4-7). In addition, short-term administration of digoxin in the setting of severe heart failure produces favorable hemodynamic changes (8,9). Proposed mechanisms for the beneficial effects of digoxin treatment in the setting of congestive heart failure include inotropic effects, alterations in systemic neurohormonal activation, modulation of baroreceptor reflexes and effects on conduction and heart rate (10). Although one or all of these effects may be operative with respect to digoxin in the setting of heart failure, the fundamental and specific effects on LV and myocyte function with long-term digoxin treatment during the progression of LV pump failure remain unknown (11). Accordingly, the overall objective of the present study was to determine the direct effects of long-term digoxin therapy on LV and myocyte contractile performance during the development of chronic LV dysfunction.

Our laboratory, as well as those of others, has demonstrated that long-term pacing-induced tachycardia in animals causes LV dilation and dysfunction (12-15). This model of long-term rapid pacing causes increased neurohormonal activity and

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signs and symptoms consistent with the clinical spectrum of congestive heart failure (14,16,17). These characteristics have resulted in the recognition of this model as an important new avenue in basic research on LV pump failure (18). In addition to changes in LV pump function and neurohormonal activity, it has also been demonstrated that fundamental defects in isolated myocyte contractile performance occur in this model of LV failure (15). Long-term digoxin treatment modulates heart rate through direct cellular effects, through modifications of autonomic neural input and through changes in myocardial sympathetic sensitivity (19), and this rate-slowness effect, in and of itself, may provide a beneficial effect in congestive heart failure, independent of the potential effects digoxin may have on LV and myocyte function. Because the model of tachycardia-induced LV dysfunction is achieved through maintaining a chronically elevated heart rate, the confounding influence of heart rate modulation, which may occur in long-term digoxin therapy, is eliminated. Therefore, this model provides a means to determine the direct effects of digoxin treatment on LV and myocyte function in the setting of chronic LV failure. Accordingly, the present study utilized this model to test the central hypothesis that long-term digoxin treatment has direct and beneficial effects on LV and myocyte function with the development of LV failure.

## Methods

The present study used a model of rapid ventricular pacing in chronically instrumented dogs that has been well described previously (20). A subset of dogs was treated with digoxin during the rapid pacing protocol. In light of the fact that digoxin is clinically given with a diuretic, furosemide was administered daily to the dogs in the rapid pacing group, with or without concomitant digoxin treatment. To assess the potential effects of digoxin in normal myocardium, a subset of control dogs was also given digoxin. After a 4-week pacing protocol, LV hemodynamic measures were studied, hearts were harvested and myocytes were isolated for contractile function and electrophysiologic studies.

**Model of pacing-induced LV pump failure.** Eighteen adult mongrel dogs of either gender (9 to 16 months of age, 15 to 25 kg, provided by Hazelton) were used in this study. The animals were chronically instrumented to measure LV and arterial pressures. In addition, a pacemaker and stimulating electrode were implanted to produce rapid right ventricular pacing. Briefly, the animals were anesthetized with thiopental (2 mg/kg body weight Pentothal, Abbot Labs), intubated and ventilated with oxygen. Maintaining a surgical plane of anesthesia with 1% to 3% isoflurane (Aurthar, Anaquest), a left thoracotomy was performed, and a shielded stimulating electrode was sutured onto the right ventricular outflow tract and connected to a programmable pacemaker previously modified for pacing rates up to 300 beats/min (Spectrax 5985, Medtronic, Inc.). A previously calibrated microtip transducer (model p5-X4, Konigsberg Instruments) was placed into the left ventricle through a small incision at the apex. The right

carotid artery was exposed and a vascular access port (model GPV, 9F, Access Technologies) was placed in the artery, advanced to the aortic arch and sutured in place for subsequent arterial blood pressure monitoring. The animals were allowed a 14-day recovery period, after which proper operation of all implanted instrumentation was confirmed. All animals used in this study were treated and cared for in accordance with the National Institutes of Health's (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council 1985, NIH publication no. 86-23).

**Experimental design.** After recovery from the surgical procedure just described, baseline LV pressure and dimensions and arterial pressure were measured. The pacemakers were then activated for rapid ventricular pacing ( $216 \pm 2$  beats/min), and 1:1 capture was confirmed by electrocardiography. The dogs were then randomly assigned to one of four protocols: 1) *Rapid pacing and digoxin*: Five dogs were given oral digoxin (0.25 mg/day) with a maintenance dose of oral furosemide (20 mg twice a day) during the 4-week pacing period. 2) *Rapid pacing only*: Five dogs were given gelatin capsule placebos and a maintenance dose of oral furosemide (20 mg twice a day) during the pacing period. 3) *Sham control*: Five dogs were instrumented and cared for in an identical manner to the groups just described, with the exception of activation of the pacemaker and drug treatment. 4) *Drug control*: Three instrumented dogs were given oral digoxin (0.25 mg/day) without rapid ventricular pacing or the additional administration of furosemide. The digoxin dose was based on previously published work in the species (21). Cardiac auscultation and an electrocardiogram (ECG) were performed frequently during the 28-day pacing protocol to ensure proper pacemaker function and the presence of 1:1 capture.

**Left ventricular function and plasma norepinephrine measurements.** Indexes of LV systolic and diastolic function were obtained at baseline and after the 28-day pacing period using simultaneously recorded pressure and echocardiographic measurements previously described (22-24). All measurements were performed at least 30 min after pacemaker deactivation, in a darkened room, with the animal resting quietly in a sling. Pressures from the fluid-filled aortic catheter were obtained using an externally calibrated transducer (Statham P23ID, Gould). The ECG and pressure waveforms were recorded using a multichannel recorder (Gould, TA4000) and digitized for subsequent analysis (PO-NE-MAH). Two-dimensional and M-mode echocardiographic studies (ATL Ultramark 7, 3.5-MHz transducer) were used to image the left ventricle from a right parasternal approach. Left ventricular volumes, cardiac output and ejection fractions were computed from the two-dimensional and M-mode echocardiographic recordings (23,24). The peak rates of positive and negative left ventricular pressure change (dp/dt), peak systolic wall stress and end-systolic wall stress were computed using methods described previously (24). Finally, LV mass was computed from the two-dimensional targeted echocardiographic recordings using previously validated methods (23).

To examine the relation between changes in plasma nor-

epinephrine levels, which may accompany changes in LV function with long-term rapid pacing with or without concomitant digoxin therapy, blood samples were drawn at the end of the study. With the animal resting quietly, 5 ml of blood was drawn from the arterial access port into tubes containing ethylenediaminetetraacetic acid (1.5 mg/ml), sodium azide (0.2 mg/ml) and aprotinin (1.15 trypsin inhibition units/ml). The blood samples were immediately centrifuged (2,000g, 10 min, 4°C), and the plasma was decanted into separate tubes, frozen in a dry ice/methanol bath and stored at -80°C until the time of assay. From these plasma samples, norepinephrine concentration was determined in duplicate using high performance liquid chromatography and normalized to pg/ml of plasma (25). In the dogs randomized to chronic digoxin therapy, plasma was also obtained for trough digoxin levels. Plasma digoxin levels were measured using a commercially available fluorescence polarization immunoassay (TDxFLx System, Abbott Laboratories).

#### **Myocyte isolation and contractile function measurements.**

Myocytes were isolated from the LV free wall using methods described by our laboratory previously (15,17,26,27). Briefly, the region of the LV free wall incorporating the left circumflex coronary artery was perfused with a collagenase solution (0.5 mg/ml, Worthington, type II; 146 U/mg) for 35 min. The tissue was then minced into 2-mm sections and gently agitated. After 15 min, the supernatant was removed and filtered and the cells were allowed to settle. Using this myocyte isolation method, a high yield of viable myocytes was routinely obtained for myocyte contractile function and electrophysiologic measurements as described in the following sections.

Isolated myocytes were placed in a thermostatically controlled chamber (37°C) fitted with a coverslip on the bottom for imaging on an inverted microscope (Sedival, Jena, Germany). Isolated myocytes were imaged using a 20× long working-distance objective. Myocyte contractions were elicited by field stimulation at 1 Hz (S11, Grass Instruments) and imaged using a charge-coupled device with a noninterlaced scan rate of 240 Hz (GPCD60, Panasonic). Myocyte motion signals were captured with the cell parallel to the video raster lines, and this video signal was input through an edge detector system (Crescent Electronics). The distance between the left and right myocyte edges was converted into a voltage signal, digitized and input to a computer (80486, Zeos International) for subsequent analysis. Stimulated myocytes were allowed a 5-min stabilization period, after which contraction data for each myocyte were recorded from a minimum of 20 consecutive contractions. Variables computed from the digitized contraction profiles included percent shortening (%), peak velocity of shortening ( $\mu\text{m/s}$ ) and peak velocity of relengthening ( $\mu\text{m/s}$ ). After collection of baseline indices of myocyte function, measurements were then repeated in the presence of 25 nmol/liter of (-) isoproterenol (17).

**Myocyte action potential measurements.** Myocytes were impaled by gently advancing microelectrodes (tip resistances 20 to 35 M $\Omega$ ) onto the sarcolemmal surface using a micromanipulator (MO-103; Narshige Instruments, Tokyo, Japan).

Myocyte action potentials were elicited by current injection through the microelectrode using 1-ms pulses at intervals of 1,000 ms. Impalements were considered stable if myocytes could be stimulated continuously for 10 min with stable rest membrane potential, action potential duration and contraction amplitude (28). Myocyte action potential signals were collected from a minimum of five consecutive contractions, digitized at 12-bit resolution at a sampling frequency of 10 kHz (AT-MIO-16; National Instruments) and stored on computer disk (80486, Zeos International). Action potential variables derived from the undifferentiated and differentiated membrane potential signals included rest membrane potential (mV), action potential amplitude (mV), action potential durations at 50% (APD<sub>50</sub>) and 90% (APD<sub>90</sub>) repolarization (ms) and the maximal action potential upstroke velocity (V/s). Action potential amplitude was computed as the difference between the peak membrane potential and the rest membrane potential. The APD<sub>50</sub> and APD<sub>90</sub> were computed as the time required for the action potential to repolarize to 50% and 10% of the action potential amplitude, respectively. Maximal action potential upstroke velocity was defined as the peak value of the differentiated membrane potential signal.

**Myocyte cross-sectional area measurements.** The left anterior descending coronary artery specimen of LV myocardium previously isolated for microscopic analysis was perfused with a buffered sodium cacodylate solution containing 2% paraformaldehyde and 2% glutaraldehyde (pH 7.4, 325 mOsm) for 20 min using a perfusion pressure of 100 mm Hg (15). Light microscopic examination at a magnification of 1,000× was performed on the perfusion-fixed LV myocardium to determine myocyte cross-sectional area. Myocytes in a cross-sectional orientation were digitized and analyzed using an image analysis system (IBAS, Kontron, Germany). Only those myocytes in which the nucleus was centrally located within the cell were digitized and analyzed to ensure that the short axis of the myocyte was perpendicular to the microscope objective. Using this approach, myocyte cross-sectional area could be determined *in situ*.

**Data analysis.** Indexes of LV and myocyte function were compared among the treatment groups using analysis of variance (ANOVA). Analysis of the morphologic data was performed using the average measurements obtained for each animal, and the groups were compared using ANOVA. For the myocyte function and electrophysiologic studies, ANOVA using a randomized block, split-plot design was employed. The treatment effects were pacing and digoxin therapy. Each dog was considered a complete block. Thus, the number of myocytes studied from each dog were considered repeated observations within each block. The summary statistics include the number of myocytes studied from each group; however, all statistical comparisons were performed on a per dog (block) basis. If ANOVA revealed significant differences, pairwise tests of individual group means were compared using Bonferroni probabilities (29). For comparisons of plasma norepinephrine and digoxin levels between groups, the Mann-Whitney rank-sum test was employed (29). All statistical procedures

**Table 1.** Changes in Left Ventricular Function With Chronic Rapid Pacing: Effects of Long-Term Digoxin Treatment

	Control Group (n = 5)	Rapid Pacing Group (n = 5)	Rapid Pacing + Digoxin Group (n = 5)
Rest heart rate (beats/min)	79 ± 4	125 ± 8*	93 ± 12
Mean arterial pressure (mm Hg)	108.4 ± 4.3	98.5 ± 2.5	98.3 ± 1.8
LV systolic pressure (mm Hg)	126.5 ± 4.9	112.2 ± 3.9*	115.2 ± 2.6
LV end-diastolic pressure (mm Hg)	9.3 ± 1.5	16.9 ± 1.0*	11.8 ± 0.7†
LV peak +dP/dt (mm Hg/s)	2,465 ± 152	1,702 ± 100*	1,916 ± 112*
LV end-diastolic volume (ml)	58.9 ± 6.6	84.4 ± 6.2*	78.6 ± 6.1*
LV ejection fraction (%)	68.3 ± 3.3	30.4 ± 1.6*	37.5 ± 2.8*†
LV peak wall stress (g/cm <sup>2</sup> )	125 ± 9	219 ± 15*	191 ± 17*
LV end-systolic wall stress (g/cm <sup>2</sup> )	111 ± 10	173 ± 6*	164 ± 5*
Cardiac output (liters/min)	4.3 ± 0.7	2.6 ± 0.2*	3.0 ± 0.3
LV mass/body weight (g/kg)	6.1 ± 0.3	6.0 ± 0.6	5.4 ± 0.3

\*p < 0.05 versus control group. †p < 0.05 versus rapid pacing group. Data presented are mean values ± SEM. dP/dt = peak rate of change in left ventricular (LV) pressure; Rapid Pacing = 28 days of right ventricular pacing at 210 beats/min; Rapid Pacing + Digoxin = rapid pacing with concomitant digoxin (0.25 mg/day).

were performed using the BMDP statistical software package (BMDP Statistical Software Inc.). Results are presented as mean ± SEM. Values of p < 0.05 were considered to be statistically significant.

## Results

Hemodynamic, echocardiographic and ECG studies were successfully performed in all experimental animals in the present study. In all of the dogs used in this study, the prepacing basal value for LV ejection fraction was 62 ± 3% and the LV end-diastolic volume was 53 ± 4 ml. Hearts were harvested and myocytes were successfully isolated from all animals at the end of the study, with no significant difference in the yield of viable myocytes obtained (76 ± 3%). In those dogs that underwent long-term digoxin therapy, plasma digoxin levels were 1.2 ± 0.2 ng/ml (range 0.6 to 3.1).

**Left ventricular function and electrocardiography: effect of rapid pacing and concomitant digoxin therapy.** Indexes of LV function in control, chronic rapid pacing and chronic rapid pacing with concomitant digoxin treatment groups are shown in Table 1. In the present study, chronic rapid pacing with the addition of daily furosemide treatment caused significant LV dilation and dysfunction. The values obtained are identical to those obtained in dogs after chronic rapid ventricular pacing without furosemide treatment (30). Therefore, the present study demonstrated that concomitant diuretic therapy during

chronic rapid pacing failed to prevent LV dilation and dysfunction. In the group of dogs treated with digoxin during chronic rapid pacing compared with the chronic rapid pacing only group, there was a small but significant increase in LV ejection fraction (p = 0.038). Left ventricular end-diastolic volume was slightly lower in the pacing group treated with digoxin compared with rapid pacing only group, but the difference did not reach statistical significance (p = 0.52). Left ventricular systolic pressure was significantly lower in the rapid pacing-only group compared with the control group. Left ventricular systolic pressure was lower in the pacing group treated with digoxin than in the control group, but this difference failed to reach statistical significance (p = 0.078). Compared with the control group, LV mass was unchanged in the chronic rapid pacing and chronic rapid pacing with concomitant digoxin administration groups. To investigate the relation between changes in LV pressure and geometry with chronic rapid pacing and with concomitant digoxin treatment, LV peak systolic and LV end-systolic wall stresses were computed. Chronic rapid pacing caused a significant increase in both LV peak and LV end-systolic wall stress. Both LV peak and end-systolic wall stresses were lower in the rapid pacing group treated with digoxin compared with the rapid pacing only group, but these differences failed to reach statistical significance (p = 0.16 and p = 0.39, respectively). Thus, concomitant digoxin administration with chronic rapid pacing improved LV pump function but did not prevent the alterations in LV geometry, which occurred with chronic rapid pacing.

In control dogs that underwent long-term digoxin administration, there was no change in LV geometry or function compared with untreated normal dogs. Specifically, there was no change in LV end-diastolic volume (73 ± 22 ml, p = 0.5) or in LV ejection fraction (66 ± 3%, p = 0.6) in control dogs treated with digoxin compared with untreated normal dogs. Similarly, there was no change in peak LV wall stress (147 ± 6 g/cm<sup>2</sup>, p = 0.14), mean arterial pressure (114.6 ± 7.9 mm Hg, p = 0.48) or the LV mass to body weight ratio (5.6 ± 0.9, p = 0.6) in control dogs treated with digoxin compared with untreated normal dogs. Thus, long-term digoxin therapy in the presence of normal myocardium had no effect on LV geometry and function.

In the chronic rapid pacing group, the plasma norepinephrine concentration was 404 ± 63 pg/ml, which was significantly elevated compared with the control group (146 ± 16 pg/ml, p < 0.05). With digoxin treatment, the plasma norepinephrine concentration was decreased from rapid pacing alone (245 ± 30 pg/ml, p < 0.05), but remained greater than that in the control group (p < 0.05). Thus, concomitant digoxin treatment modulated the increase in systemic norepinephrine levels which occurred with chronic rapid pacing.

Electrocardiographic variables measured at the end of the study are shown in Table 2. All ECGs were recorded at an ambient rest heart rate at least 30 min after pacemaker deactivation (paced dogs only). The RR interval, which was computed as a measure of the rest heart rate, was increased in the chronic rapid pacing with concomitant digoxin treatment

**Table 2.** Changes in Electrocardiographic Variables With Chronic Rapid Pacing: Effects of Long-Term Digoxin Treatment

	Control Group (n = 5)	Rapid Pacing Group (n = 5)	Rapid Pacing + Digoxin Group (n = 5)
RR interval (ms)	701 ± 34	524 ± 29*	756 ± 87†
PR interval (ms)	111 ± 3	126 ± 3*	157 ± 4*†
QRS duration (ms)	63 ± 2	72 ± 3*	71 ± 1*
QTc interval (ms)	292 ± 4	331 ± 6*	269 ± 9†

\*p < 0.05 versus control group. †p < 0.05 versus rapid pacing group. Data presented are mean value ± SEM. QTc = corrected QT interval for heart rate using Bazett's formula; Rapid Pacing = 28 days of right ventricular pacing at 210 beats/min; Rapid Pacing + Digoxin = rapid pacing with concomitant digoxin (0.25 mg/day).

group compared with the chronic rapid pacing only group. The PR interval was significantly increased in the chronic rapid pacing with concomitant digoxin therapy group compared with the control and rapid pacing only groups. QRS duration was prolonged in both the chronic rapid pacing and the chronic pacing with digoxin treatment groups compared with that in the control group. The corrected QT interval (QTc), which was prolonged by chronic rapid pacing, was decreased in the rapid pacing with digoxin group compared with the rapid pacing only group. Thus, the chronic rapid pacing and chronic rapid pacing with concomitant digoxin treatment groups displayed differential effects with respect to ECG variables when compared with the control group.

**Isolated myocyte geometry and contractile function: effect of rapid pacing and concomitant digoxin therapy.** A summary of isolated myocyte rest length and contractile function is shown in Table 3. Isolated myocyte rest length increased compared with control values after 4 weeks of rapid pacing in both groups of paced dogs, but was significantly lower in the pacing group with concomitant digoxin treatment. To more carefully examine the structural basis for the changes in LV and myocyte geometry in the rapid pacing groups, perfusion-fixed myocardial sections were examined using light microscopy. Myocyte cross-sectional area was significantly lower in the rapid pacing only group compared with the control group ( $272 \pm 4 \mu\text{m}^2$  vs.  $318 \pm 4 \mu\text{m}^2$ , respectively,  $p < 0.001$ ). Rapid pacing with concomitant digoxin treatment caused an increase in myocyte cross-sectional area over the rapid pacing only values ( $302 \pm 4 \mu\text{m}^2$ ,  $p < 0.001$ ). However, this value remained significantly lower than the control group value ( $p = 0.01$ ).

Representative contraction profiles of isolated myocytes taken from animals in the control, rapid pacing and rapid pacing with concomitant digoxin groups are shown in Figure 1. Compared with the control group, isolated myocyte percentage and velocity of shortening were significantly reduced with chronic rapid pacing and with chronic rapid pacing with concomitant digoxin treatment. However, compared with the rapid pacing only group, percent shortening and velocity of shortening were each 24% greater in the rapid pacing with concomitant digoxin administration group. Similarly, velocity of relengthening was diminished in both pacing groups com-

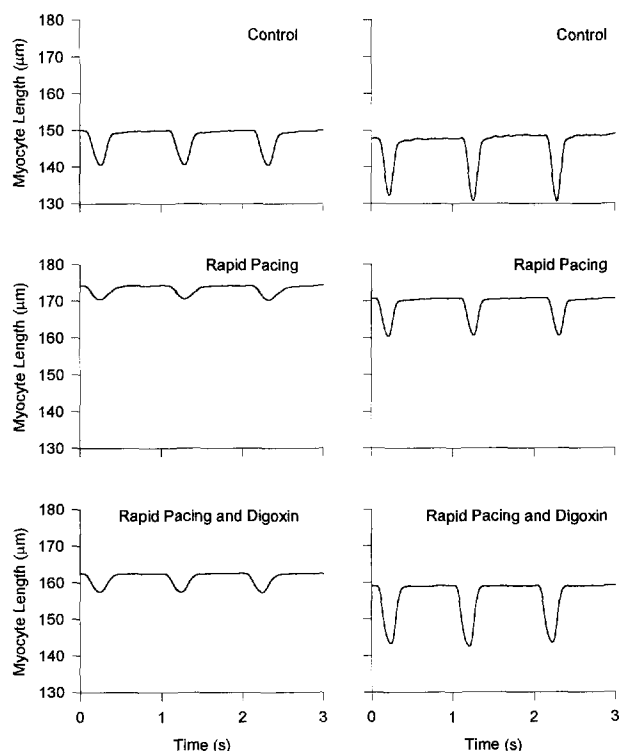
**Table 3.** Isolated Myocyte Contractile Performance With Chronic Rapid Pacing: Effects of Long-Term Digoxin Treatment

	Baseline	Isoproterenol (25 nmol/liter)	Calcium (8 mmol/liter)
Rest length ( $\mu\text{m}$ )			
Control	152 ± 1	148 ± 1‡	147 ± 4
Control + digoxin	154 ± 1	147 ± 2‡	145 ± 3
Rapid pacing	172 ± 1*	163 ± 1*‡	169 ± 5*
Rapid pacing + digoxin	162 ± 1*†	153 ± 1*‡	153 ± 2‡‡
Percent shortening (%)			
Control	4.2 ± 0.1	10.3 ± 0.2‡	10.4 ± 0.8‡
Control + digoxin	4.2 ± 0.1	10.2 ± 0.2‡	9.8 ± 0.7‡
Rapid pacing	2.5 ± 0.1*	7.2 ± 0.2*‡	6.9 ± 0.7*‡
Rapid pacing + digoxin	3.1 ± 0.1*†	9.5 ± 0.2*‡	6.3 ± 0.5*‡
Shortening velocity ( $\mu\text{m/s}$ )			
Control	59.3 ± 1.0	187.9 ± 5.8‡	160.9 ± 18.2‡
Control + digoxin	56.6 ± 1.5	189.9 ± 6.9‡	154.4 ± 15.1‡
Rapid pacing	36.8 ± 1.0*	132.0 ± 4.2*‡	111.1 ± 13.4*‡
Rapid pacing + digoxin	45.7 ± 0.9*†	170.7 ± 5.6*‡	101.1 ± 10.7*‡
Relengthening velocity ( $\mu\text{m/s}$ )			
Control	60.5 ± 1.2	197.5 ± 5.9‡	186.8 ± 20.6‡
Control + digoxin	57.9 ± 1.9	187.1 ± 7.4‡	157.4 ± 14.1‡
Rapid pacing	32.5 ± 1.0*	124.3 ± 4.4*‡	111.8 ± 13.6*‡
Rapid pacing + digoxin	45.2 ± 1.1*†	168.9 ± 6.4*‡	117.6 ± 13.7*‡
No. of cells			
Control	422	240	30
Control + digoxin	248	137	26
Rapid pacing	395	291	31
Rapid pacing + digoxin	452	226	59

\*p < 0.05 versus control group. †p < 0.05 versus rapid pacing group. ‡p < 0.05 versus baseline. Data presented are mean value ± SEM. Control + digoxin = sham controls with concomitant digoxin (0.25 mg/day); Rapid pacing = 28 days of right ventricular pacing at 210 beats/min; Rapid pacing + digoxin = rapid pacing with concomitant digoxin (0.25 mg/day).

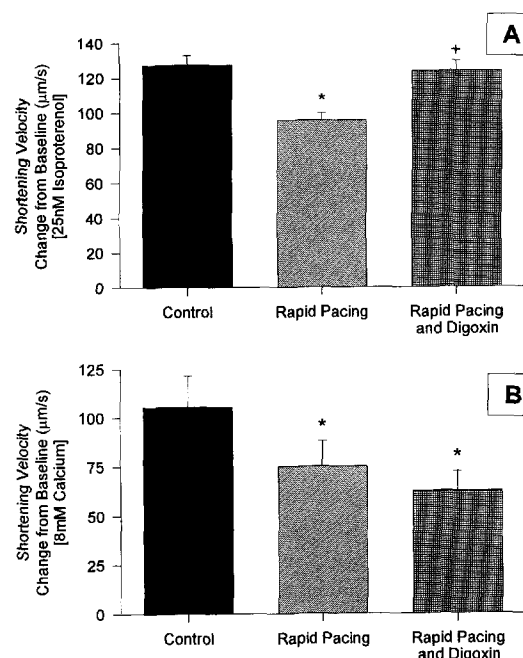
pared with the control group. In the pacing with digoxin treatment group, velocity of relengthening was 39% greater than in the rapid pacing only group. In the drug control group, there were no differences in myocyte length or indexes of myocyte contractile function compared with the group of untreated normal dogs. Thus, consistent with past reports (15,17,26,30), chronic rapid pacing resulted in reduced myocyte contractile performance. This study demonstrated that concomitant digoxin treatment during chronic rapid pacing improved myocyte contractile performance.

In the next series of studies, the capacity of isolated myocytes to respond to an inotropic stimulus was examined after exposure to either the beta-adrenergic agonist isoproterenol or increased extracellular calcium. The results from this series of experiments are shown in Table 3. Isolated myocyte contractile performance in the presence of isoproterenol or calcium was increased compared with baseline (no beta-adrenergic or calcium stimulation) values for all three groups. However, isolated myocyte function was significantly lower with isoproterenol or calcium in the chronic rapid pacing group compared with the control group. Compared with the rapid pacing only group, concomitant digoxin treatment increased myocyte contractile function in the presence of beta-



**Figure 1.** Representative myocyte contraction profiles in control, chronic rapid pacing and rapid pacing with concomitant digoxin groups at baseline (left) and in the presence 25 nmol/liter of isoproterenol (right). Compared with the control value, myocyte length was increased with both rapid pacing and rapid pacing with digoxin. Percent and velocity of shortening were diminished in the rapid pacing and the rapid pacing and digoxin groups compared with the control group. However, both percent and velocity of myocyte shortening were greater with rapid pacing and concomitant digoxin compared with rapid pacing alone. In the presence of isoproterenol, myocyte contraction amplitude was increased in all groups compared with baseline values. However, both myocyte percent and velocity of shortening were diminished in the rapid pacing and rapid pacing with digoxin groups compared with the control group. After beta-adrenergic receptor stimulation, myocyte percent and velocity of shortening were greater with rapid pacing with concomitant digoxin than with rapid pacing alone. Indexes of myocyte contractile function at baseline and after beta-adrenergic receptor stimulation are summarized in Table 3.

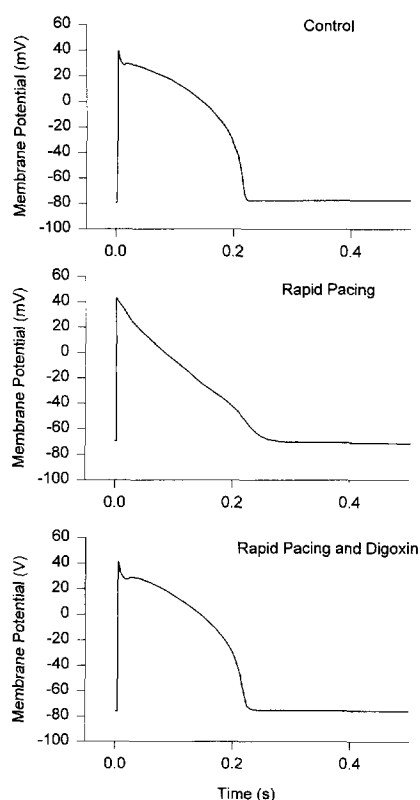
adrenergic receptor stimulation. In contrast, myocyte contractile function in the presence of increased extracellular calcium was not increased in the digoxin-treated group compared with the rapid pacing only group. In light of the differences in baseline myocyte function in the control, rapid pacing and rapid pacing with digoxin treatment groups, the magnitude of the response to inotropic stimulation was examined as the absolute increase in velocity of shortening. This analysis was performed using paired values for shortening velocity in those myocytes which were exposed to either isoproterenol or increased extracellular calcium. The results of this analysis are summarized in Figure 2. Compared with the chronic rapid pacing group, concomitant digoxin treatment caused improved myocyte contractile performance with beta-adrenergic recep-



**Figure 2.** The absolute change from baseline for myocyte shortening velocity with 25 nmol/liter of isoproterenol (A) or 8 mmol/liter of extracellular calcium (B). Beta-adrenergic and calcium responsiveness was diminished in the chronic rapid pacing group compared with the control group. With rapid pacing and digoxin, beta-adrenergic responsiveness was normalized to the control value. In contrast, calcium responsiveness with rapid pacing and digoxin remained significantly diminished. \* $p < 0.05$  versus control; + $p < 0.05$  versus rapid pacing.

tor stimulation, but did not improve myocyte contractile function with exposure to increased extracellular calcium.

**Isolated myocyte electrophysiology: effect of rapid pacing and concomitant digoxin therapy.** To determine the effect of rapid pacing with or without concomitant digoxin treatment on isolated myocyte electrophysiologic measures, intracellular action potentials were recorded. Representative action potentials from each group are shown in Figure 3, and the data from this series of experiments are summarized in Table 4. In the chronic rapid pacing group compared with the control group, isolated myocyte rest membrane potential was more positive, maximal upstroke velocity decreased and action potential duration increased. With concomitant digoxin and rapid pacing, rest membrane potential normalized. Further, maximal upstroke velocity increased and action potential duration decreased compared with the pacing only group. Thus, chronic rapid pacing caused significant changes in isolated myocyte action potential characteristics. Concomitant digoxin treatment during the rapid pacing protocol normalized these important characteristics of isolated myocyte electrophysiology. In control dogs that underwent chronic digoxin treatment (drug control group), there was no change in isolated myocyte action potential characteristics. In the drug control group compared with the group of untreated normal dogs, there was no change in rest membrane potential or any other myocyte action potential variable. Thus, chronic digoxin administration



**Figure 3.** Representative isolated myocyte action potentials in the control, chronic rapid pacing and rapid pacing with concomitant digoxin groups. In the rapid pacing group, rest membrane potential was more positive, maximal upstroke velocity was decreased and action potential duration was prolonged compared with the control group. With rapid pacing and digoxin, action potential variables were normalized. Indexes of action potential data for the three groups are summarized in Table 4.

in the presence of normal myocardium had no effect on isolated myocyte electrophysiologic measures.

## Discussion

Congestive heart failure is a significant cause of morbidity and mortality, and the prevalence is increasing (18). Thus,

attempts to discern potential mechanisms of current therapeutic interventions in the setting of heart failure remain a major focus of cardiovascular research. The cardiac glycoside preparation digoxin is a common therapeutic agent used in patients with heart failure. However, the mechanism of action for chronic digoxin therapy in the setting of LV failure remains unclear (11). Accordingly, the present study addressed these issues in a model of tachycardia-induced LV dysfunction with and without concomitant digoxin therapy. There were several important and unique findings of this study. First, concomitant digoxin with chronic rapid pacing improved LV and myocyte function, compared with chronic pacing alone. Second, concomitant digoxin with chronic rapid pacing normalized myocyte beta-adrenergic responsiveness. Third, concomitant digoxin treatment with chronic rapid pacing normalized abnormalities in myocyte action potential characteristics. Thus, this study provides unique evidence to suggest that the cellular basis for improved LV pump function with digoxin treatment during the progression of LV failure has a direct and beneficial effect on myocyte contractile function and electrophysiologic measures.

Although there is an historic precedent for the use of digitalis glycoside agents in the setting of congestive heart failure (1,2), only recently have clinical trials demonstrated the benefits of this therapeutic modality. Specifically, large multicenter trials have demonstrated that digoxin administration improves symptoms and echocardiographic indexes of LV function in patients with congestive heart failure (4,5,7,8). Other clinical trials have demonstrated that for patients with LV dysfunction digoxin is the only available positive inotropic agent that reduces symptoms without a demonstrable increase in mortality (6,31). Therefore, there is reasonable clinical evidence to support the use of digoxin in patients with LV dysfunction, but the mechanisms underlying the beneficial effects remain unclear. To our knowledge, the present study is the first to examine the direct effects of digoxin therapy on LV and myocyte function with the development of chronic LV failure. The findings of the present study provide direct evidence that a contributory mechanism for improved LV

**Table 4.** Myocyte Action Potential Characteristics With Chronic Rapid Pacing: Effects of Long-Term Digoxin Treatment

	Control Group	Control + Digoxin Group	Rapid Pacing Group	Rapid Pacing + Digoxin Group
Rest membrane potential (mV)	$-80.6 \pm 0.8$	$-79.8 \pm 1.3$	$-74.0 \pm 1.1^*$	$-77.3 \pm 1.2$
Maximal upstroke velocity (V/s)	$163 \pm 9$	$156 \pm 8$	$135 \pm 6^*$	$155 \pm 12^\dagger$
Action potential amplitude (mV)	$107 \pm 2$	$113 \pm 2$	$106 \pm 2$	$109 \pm 2^\dagger$
APD <sub>50</sub> (ms)	$162 \pm 6$	$169 \pm 10$	$154 \pm 8$	$160 \pm 14$
APD <sub>90</sub> (ms)	$216 \pm 6$	$221 \pm 10$	$247 \pm 10^*$	$219 \pm 12^\dagger$
No. of cells	21	20	21	14

\*p < 0.05 versus control group. †p < 0.05 versus rapid pacing group. Data presented are mean value ± SEM. APD<sub>50</sub> = action potential duration at 50% repolarization; APD<sub>90</sub> = action potential duration at 90% repolarization; Control + Digoxin = sham control group with concomitant digoxin (0.25 mg/day); Rapid Pacing = 28 days of right ventricular pacing at 210 beats/min; Rapid Pacing + Digoxin = rapid pacing with concomitant digoxin (0.25 mg/day).

pump function with chronic digoxin therapy has direct beneficial effects on myocyte contractile processes.

**Left ventricular geometry and function.** In the present study, concomitant digoxin treatment with chronic rapid pacing caused a slight but significant increase in LV ejection fraction, but did not prevent the progressive LV dilation, which invariably occurs with chronic rapid pacing (15,17,20,24,26,30). The increased LV ejection fraction observed in the present study with chronic digoxin therapy is consistent with clinical reports (6,7,32). In addition, Sabbah et al. (21) recently reported that digoxin therapy in dogs with progressive LV dysfunction due to microembolization improved LV ejection fraction, but that progressive LV dilation was not prevented. In the present study, digoxin therapy with chronic rapid pacing improved LV ejection fraction but did not prevent LV dilation. Although not statistically significant, both LV peak and end-systolic wall stresses were lower in the rapid pacing group treated with digoxin compared with the rapid pacing only group. Thus, changes in LV afterload may have contributed to the increased LV ejection fraction in the rapid pacing group treated with digoxin. However, a more likely contributory factor toward the improvement in LV ejection fraction was the improvement in myocyte contractile function in the rapid pacing group treated with digoxin.

Digoxin treatment exerts both direct and indirect effects on cardiac electrophysiologic measures, and some of these effects are reflected in ECG changes (19). In the present study, ECG changes that occurred with the development of rapid pacing-induced LV pump failure included an increased heart rate, prolonged QRS duration and prolonged QTc interval. Concomitant digoxin therapy with chronic rapid pacing reduced the ambient rest heart rate, prolonged the PR interval and shortened the QTc interval. Thus, concomitant digoxin treatment caused specific changes in electrocardiography that are consistent with clinical observations (19). Importantly, these measurements were obtained at steady state ambient heart rate at least 30 min after pacemaker deactivation. However, in the present study, the induction of LV pump failure was performed using a constant, pacing-induced, ventricular rate. This chronic pacing rate probably removed any influence that chronic digoxin therapy would have on myocardial activation processes. The use of this model of chronic LV dysfunction effectively removed the potential confounding influence of heart rate modulation by chronic digoxin therapy. Therefore, the beneficial effects of chronic digoxin therapy on LV and myocyte function in this model of LV dysfunction were not related to effects of digoxin on heart rate control or myocardial activation processes.

**Isolated myocyte geometry and contractile function.** Consistent with past reports from our laboratory (15,17,26), isolated myocyte length was increased and myocyte cross-sectional area was decreased with rapid pacing-induced LV failure. Concomitant digoxin therapy with chronic rapid pacing improved but did not normalize isolated myocyte length and myocyte cross-sectional area. Thus, a structural basis for the persistent LV dilation observed with chronic rapid pacing and

concomitant digoxin therapy was continued abnormalities in myocyte geometry. Past studies have provided evidence that potential mechanisms for the LV and myocyte remodeling with chronic rapid pacing are myocyte remodeling, realignment of myocytes within the LV free wall and an absolute reduction in the number of myocytes (33,34). For example, in a recent report by Kajstura et al. (34), a significant loss in myocytes within the LV free wall was reported to occur after chronic ventricular pacing tachycardia in dogs. Thus, an important contributory event toward the changes in LV myocardial remodeling with pacing-induced failure may be myocyte dropout, or apoptosis. Although the specific cellular mechanisms responsible for the LV remodeling that occurs with chronic rapid pacing remain to be established, the results from the present study demonstrated that digoxin therapy did not prevent the changes in LV and myocyte geometry, which invariably occur in this model of LV failure.

**Myocyte beta-adrenergic and calcium response.** To examine the capacity of myocytes to respond to an inotropic stimulus, myocyte contractile function was examined after exposure to the beta-adrenergic agonist isoproterenol. Consistent with past reports, chronic rapid pacing caused reduced myocyte beta-adrenergic responsiveness (15,17). Concomitant digoxin treatment with rapid pacing normalized myocyte beta-adrenergic responsiveness. It has been proposed that, similar to clinical findings (35), possible mechanisms for diminished beta-adrenergic responsiveness with rapid pacing-induced LV pump failure include elevated circulating catecholamines with subsequent beta-receptor downregulation, alterations in the guanine nucleotide-binding regulatory complex and abnormalities in cyclic AMP production (13,14,17). In the present study, chronic rapid pacing increased plasma norepinephrine levels. Concomitant digoxin with chronic rapid pacing attenuated the increase in circulating norepinephrine levels. Past studies have shown that digoxin directly attenuates increased sympathetic activity present in clinical heart failure (36). Further, it has been demonstrated that digoxin can directly decrease the sympathetic efferent activity that occurs in pacing-induced LV pump dysfunction through a direct increase in baroreceptor sensitivity (37). Therefore, the reduced levels of plasma norepinephrine which were observed with concomitant digoxin and chronic rapid pacing are probably due to modulation of sympathetic efferent activity. More importantly, a potential contributory mechanism for the improved myocyte beta-adrenergic response with concomitant digoxin therapy in the setting of rapid pacing-induced LV dysfunction is a reduction in circulating catecholamines, and subsequent improved beta-adrenergic transduction. However, concomitant digoxin therapy with chronic rapid pacing did not return myocyte beta-adrenergic responsiveness to control levels. Our laboratory and those of others have demonstrated that the abnormalities in beta-adrenergic receptor transduction and responsiveness with pacing-induced LV failure were associated with alterations in the guanine nucleotide-binding regulatory protein complex (G-protein complex) (13,14,17,30). Furthermore, the development of cardiomyopathic disease has been demon-



strated to cause alterations in beta-adrenergic receptor subtype expression (35). Potential mechanisms for the continued reduction in myocyte beta-adrenergic responsiveness with concomitant digoxin treatment and rapid pacing include abnormalities in subtype expression or in the G-protein complex. Future studies that more carefully examine specific changes in beta-adrenergic receptor density and transduction with concomitant digoxin treatment and the progression of pacing LV failure would be appropriate.

The cardiac glycosides act by binding to and blocking the action of the membrane bound  $\text{Na}^+, \text{K}^+$ -ATPase. This enzyme hydrolyzes ATP in order to transport sodium into the extracellular space and to transport potassium into the cell. The  $\text{Na}^+, \text{K}^+$ -ATPase is therefore a major contributor to cellular ionic homeostasis and maintenance of the rest membrane potential. The inotropic action of cardiac glycosides is likely a result of  $\text{Na}^+, \text{K}^+$ -ATPase inhibition, which results in a slight increase in intracellular sodium concentration ( $[\text{Na}^+]_i$ ). This increased  $[\text{Na}^+]_i$  is then available as a substrate for the sarcolemmal  $\text{Na}^+/\text{Ca}^{2+}$  exchange system, resulting in increased  $[\text{Ca}^{2+}]_i$  and increased contractility (38,39). In the present study, chronic rapid pacing caused diminished myocyte contractile responsiveness to increased extracellular calcium. This was not an unexpected finding, because abnormalities in calcium homeostasis and myofilament sensitivity to calcium have been reported with pacing-induced heart failure (15,40-42). Thus, the blunted response to increased extracellular calcium with rapid pacing is probably an exacerbation of preexisting abnormalities in intracellular calcium regulation. In the present study, concomitant digoxin treatment with chronic rapid pacing failed to normalize myocyte responsiveness to increased extracellular calcium. This observation suggests that persistent defects in myocyte calcium homeostasis occurred with concomitant digoxin therapy in this model of chronic LV pump failure. An important intracellular mechanism of action with beta-receptor activation is increased calcium availability. Thus, the uncorrected defect in myocyte calcium response with rapid pacing and digoxin treatment is also a likely contributory factor toward persistently diminished myocyte beta-adrenergic response. In the present study, chronic digoxin treatment and rapid pacing improved myocyte beta-adrenergic response to a greater degree than the calcium response. This observation is likely related to the mechanism by which beta-adrenergic stimulation normally increases myocyte inotropic state, specifically cyclic AMP-dependent phosphorylation of regulatory proteins (38). Therefore, the difference in beta-adrenergic and calcium responsiveness with digoxin and chronic rapid pacing is likely due to improvements in phosphorylation capacity rather than improvements in calcium homeostatic processes.

**Isolated myocyte electrophysiology.** Ionic currents across the sarcolemma during the myocyte action potential are a determinant of myocyte excitation-contraction processes (38). Previous studies have demonstrated specific alterations in myocyte action potential characteristics with chronic rapid pacing in pigs, and these changes were directly associated with alterations in myocyte contractile function (28). In the present

study, chronic rapid pacing caused specific changes in isolated myocyte action potential characteristics. These changes included more positive rest membrane potential, decreased maximal upstroke velocity and increased action potential duration. Chronic digoxin therapy with rapid pacing normalized these action potential characteristics. To our knowledge, the present study is the first to examine the specific changes in ventricular myocyte action potential characteristics with chronic digoxin therapy in the setting of pacing-induced LV pump failure. It is difficult to directly relate the action potential findings to the ECG data, which were obtained *in vivo*. Nevertheless, the improvements in action potential characteristics which were observed in digoxin therapy with chronic rapid pacing probably contributed to improved myocyte contractile function. In the present study, isolated myocyte contractile function and electrophysiologic characteristics were measured 4 to 8 h after removal of the heart and more than 36 h after the last digoxin dose. Thus, the myocyte contractile function and action potential data obtained in the present study reflect the chronic effects of digoxin treatment with chronic rapid pacing rather than the direct effect of digoxin on action potential characteristics. Moreover, the time to 50% dissociation of ouabain bound to sarcolemmal vesicles has been previously reported by this laboratory to be approximately 77 min (26). Therefore, it is unlikely that the myocyte contractile function and action potential data were affected by digoxin bound to the sarcolemma of the isolated myocytes.

In the clinical setting, digoxin is routinely administered simultaneously with chronic diuretic therapy (6,7,32). In the present study, all experimental animals that underwent rapid pacing received daily furosemide treatment. With chronic rapid pacing alone, LV function and isolated myocyte contractile function were identical to values previously published from this laboratory in animals with rapid pacing-induced LV pump dysfunction (20,30). Thus, chronic diuretic therapy did not influence the changes in LV and myocyte geometry and function that occur with the development of rapid pacing-induced LV pump dysfunction. To examine the effect of digoxin treatment on LV and myocyte function in normal myocardium, a subset of control dogs was treated with digoxin. With digoxin treatment in normal dogs, LV dimension and ejection fraction and isolated myocyte length shortening velocity were unchanged from control values. Thus, chronic digoxin treatment in normal myocardium had no effect on LV and myocyte geometry and function.

**Limitations.** The present study demonstrated that digoxin has direct beneficial effects on LV and myocyte geometry, function and electrophysiologic measures but the fundamental mechanisms responsible for these improvements were not addressed. Specifically, important sarcolemmal enzyme systems such as  $\text{Na}^+, \text{K}^+$ -ATPase and the  $\text{Na}^+-\text{Ca}^{2+}$  exchanger were not examined. Furthermore, it is well recognized that chronic therapeutic modalities that modulate receptor activities are associated with alterations in receptor number and transduction. Our laboratory has previously demonstrated that rapid pacing-induced LV pump dysfunction causes reduced

glycoside receptor density and  $\text{Na}^+/\text{K}^+$ -ATPase activity (26). A recent report (43) did not detect a change in human LV  $\text{Na}^+/\text{K}^+$ -ATPase concentration with chronic digitalization, but the effects of chronic digoxin therapy with respect to  $\text{Na}^+/\text{K}^+$ -ATPase density and function in this model of LV pump dysfunction remain to be established. Based on the findings of the present study, future studies that quantitate glycoside receptor binding and  $\text{Na}^+/\text{K}^+$ -ATPase hydrolytic activity with chronic digoxin therapy in this model of chronic LV dysfunction may be appropriate. In the clinical setting, digoxin therapy is initiated after the development of signs and symptoms of LV dysfunction. It remains unclear from the present study whether digoxin therapy prevents the continued deterioration in LV and myocyte function in the setting of preexisting LV dysfunction. Thus, the time-dependent effects of digoxin therapy in this model of LV dysfunction remain to be established. In light of the fact that consistent time-dependent changes in LV function occur with chronic rapid pacing (13,19), this model of LV dysfunction would be well suited to study the time-dependent effects of digoxin therapy.

**Conclusions.** Although digoxin historically has been used in the treatment of heart failure, the fundamental mechanisms responsible for the clinical benefit of this therapeutic modality remain unclear. Accordingly, the present study examined the effects of chronic digoxin therapy on both ventricular and isolated myocyte function in an experimental model of chronic LV dysfunction. Digoxin treatment improved LV pump function, enhanced isolated myocyte contractile performance and beta-adrenergic responsiveness and normalized myocyte action potential characteristics. This study provides unique evidence that the fundamental basis for improved LV pump function with digoxin treatment in chronic heart failure has a direct beneficial effect on myocyte contractile function and electrophysiologic measures.

## References

1. Withering W. An account of the foxglove and some of its medical uses, with practical remarks on dropsy, and other diseases. Birmingham, UK: M. Sweeney, 1785.
2. Christian HA. Digitalis effects in chronic cardiac cases with regular rhythm in contrast to auricular fibrillation. *Med Clin North Am* 1922;5:1173-90.
3. Kelly RA. Cardiac glycosides and congestive heart failure. *Am J Cardiol* 1990;65:10E-16E.
4. Lee DC, Johnson RA, Bingham JB, et al. Heart failure in outpatients: a randomized trial of digoxin versus placebo. *N Engl J Med* 1982;306:699-705.
5. Guyatt GH, Sullivan MJ, Fallen EL, et al. A controlled trial of digoxin in congestive heart failure. *Am J Cardiol* 1988;61:371-5.
6. DiBianco R, Shabetai R, Kostuk W, Moran J, Schlant RC, Wright R, for the Milrinone Multicenter Trial Group. A comparison of oral milrinone, digoxin, and their combination in the treatment of patients with chronic heart failure. *N Engl J Med* 1989;320:677-83.
7. Packer M, Gheorghiade M, Young JB, et al., for the RADIANCE study. Withdrawal of digoxin from patients with chronic heart failure treated with angiotensin-converting enzyme inhibitors. *N Engl J Med* 1993;329:1-7.
8. Gheorghiade M, St. Clair J, St. Clair C, Beller GA. Hemodynamic effects of intravenous digoxin in patients with severe heart failure initially treated with diuretics and vasodilators. *J Am Coll Cardiol* 1987;9:849-57.
9. Ribner HS, Plucinski DA, Hsieh AM, et al. Acute effects of digoxin on total systemic vascular resistance in congestive heart failure due to dilated cardiomyopathy: a hemodynamic-hormonal study. *Am J Cardiol* 1985;56:896-904.
10. Gheorghiade M, Ferguson D. Digoxin—a neurohormonal modulator in heart failure? *Circulation* 1991;84:2181-6.
11. Dec WG. Evolving strategies for the outpatient management of advanced heart failure. *ACC Curr J Rev* 1995;57-60.
12. Coleman HN, Taylor RR, Pool PE, et al. Congestive heart failure following chronic tachycardia. *Am Heart J* 1971;81:790-8.
13. Kuchi K, Shannon RP, Komamura K, et al. Myocardial  $\beta$ -adrenergic receptor function during the development of pacing induced heart failure. *J Clin Invest* 1993;91:907-14.
14. Roth DA, Kazushi U, Helmer GA, Hammond HK. Downregulation of cardiac guanosine 5'-triphosphate binding proteins in right and left ventricle in pacing induced congestive heart failure. *J Clin Invest* 1993;91:939-49.
15. Spinale FG, Fulbright BM, Mukherjee R, et al. Relationship between ventricular and myocyte function with tachycardia induced cardiomyopathy. *Circ Res* 1992;71:174-87.
16. Margulies KB, Heublein DM, Perrella MA, Burnett JC. ANF-mediated renal cGMP generation in congestive heart failure. *Am J Physiol* 1991;260:F562-F568.
17. Spinale FG, Tempel GE, Mukherjee R, et al. Cellular and molecular changes in the  $\beta$ -adrenergic receptor system with supraventricular tachycardia induced cardiomyopathy. *Cardiovasc Res* 1994;28:1243-50.
18. Lenfant C. Report of the task force on research in heart failure. *Circulation* 1994;90:1118-23.
19. Hoffman BF, Bigger JT. Digitalis and allied cardiac glycosides. In: Gilman AG, Rall TW, Nies AS, Taylor P, editors. *Goodman and Gilman's The Pharmacologic Basis of Therapeutics*. 8th ed. Elmsford (NY): Pergamon Press, 1990:814-39.
20. Spinale FG, Holzgreffe HH, Mukherjee R, et al. LV and myocyte structure and function after early recovery from tachycardia-induced cardiomyopathy. *Am J Physiol* 1995;268:H836-H847.
21. Sabbah HN, Shimoyama H, Kono T, et al. Effects of long-term monotherapy with enalapril, metoprolol, and digoxin on the progression of left ventricular dysfunction and dilation in dogs with reduced ejection fraction. *Circulation* 1994;89:2852-9.
22. Laurenceau JL, Malergue MC. *The Essentials in Echocardiography, M-Mode and 2-Dimensional Imaging*. Norwell (MA): Martinus Nijhoff, 1981:64-70.
23. Zile MR, Tanaka R, Lindroth JR, Spinale FG, Carabello BA, Mirsky I. Left ventricular volume determined echocardiographically by using a constant LV epicardial long axis to short axis dimension ratio throughout the cardiac cycle. *J Am Coll Cardiol* 1992;20:986-93.
24. Tomita M, Spinale FG, Crawford FA, Zile MR. Changes in left ventricular volume, mass and function during development and regression of supraventricular tachycardia induced cardiomyopathy: disparity between recovery of systolic vs diastolic function. *Circulation* 1991;83:635-44.
25. Goldstein DS, Feuerstein G, Izzo HL, Kopin IJ, Keiser H. Validity and reliability of liquid chromatography with electrochemical detection for measuring plasma levels of norepinephrine and epinephrine in man. *Life Sci USA* 1986;38:265-9.
26. Spinale FG, Clayton C, Tanaka R, et al. Myocardial  $\text{Na}^+/\text{K}^+$ -ATPase in tachycardia induced cardiomyopathy. *J Mol Cell Cardiol* 1992;24:277-94.
27. Mukherjee R, Hewett K, Crawford FA, Spinale FG. Relation between dynamic cellular and sarcomere contractile properties from the same cardiocyte. *J Appl Physiol* 1993;74:2023-33.
28. Mukherjee R, Hewett KW, Spinale FG. Myocyte electrophysiological properties following the development of supraventricular tachycardia induced cardiomyopathy. *J Mol Cell Cardiol* 1995;27:1333-48.
29. Steel RGD, Torrie JH. *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd ed. New York: McGraw-Hill, 1980.
30. Spinale FG, Holzgreffe HH, Mukherjee R, et al. Angiotensin converting enzyme inhibition and the progression of congestive cardiomyopathy: effects on left ventricular and myocyte structure and function. *Circulation* 1995;92:562-78.
31. Kelly RA, Smith TW. Digoxin in heart failure: implications of recent trials. *J Am Coll Cardiol* 1993;22:107A-112A.
32. Captopril Digoxin Multicenter Research Group. Comparative effects of therapy with captopril and digoxin in patients with mild to moderate heart failure. *JAMA* 1988;259:539-44.
33. Spinale FG, Zellner JL, Tomita M, Crawford FA, Zile MR. Relationship

- between ventricular and myocyte remodelling with the development and regression of supraventricular tachycardia induced cardiomyopathy. *Circ Res* 1991;69:1058-67.
34. Kajstura J, Zhang Z, Liu Y, et al. The cellular basis of pacing induced dilated cardiomyopathy. Myocyte cell loss and myocyte cellular reactive hypertrophy. *Circulation* 1995;92:2306-17.
  35. Bristow MR, Ginsburg R, Umans V, et al.  $\beta_1$  and  $\beta_2$  adrenergic receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective  $\beta_1$ -receptor down regulation in heart failure. *Circ Res* 1986;59:297-309.
  36. Ferguson DW, Berg WJ, Sanders JS, Roach PJ, Kempf JS, Kienzie MG. Sympathoinhibitory responses to digitalis glycosides in heart failure patients. *Circulation* 1989;80:65-77.
  37. Wang W, Chen J-S, Zucker IH. Carotid sinus baroreceptor sensitivity in experimental heart failure. *Circulation* 1990;81:1959-66.
  38. Katz AM. *Physiology of the Heart*. 2nd ed. New York: Raven Press, 1992.
  39. Eisner DA, Smith TW. The Na-K pump and its effectors in cardiac muscle. In: Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE, editors. *The Heart and Cardiovascular System*. 2nd ed. Raven Press: New York, 1992: 863-902.
  40. Cory CR, McCutcheon LJ, O'Grady M, Pang AW, Geiger JD, O'Brien PJ. Compensatory down regulation of myocardial Ca channel in sarcoplasmic reticulum from dogs with heart failure. *Am J Physiol* 1993;264:H926-H937.
  41. Woolf MR, Whitesell LF, Moss RL. Calcium sensitivity of isometric tension is increased in canine experimental heart failure. *Circ Res* 1995;76:781-9.
  42. Zile MR, Mukherjee R, Clayton C, Kato S, Spinale FG. Effects of chronic supraventricular pacing tachycardia on relaxation rate in isolated cardiac muscle cells. *Am J Physiol* 1995;268:H2104-H2113.
  43. Schmidt TA, Holm-Nielsen P, Kjeldsen K. No upregulation of digitalis glycoside receptor ( $\text{Na}^+$ ,  $\text{K}^+$ -ATPase) concentration in human heart left ventricle samples obtained at necropsy after long term digitalisation. *Cardiovasc Res* 1991;25:684-91.